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(54) Process for solubilizing polyanionic bacterial polysaccharides in aprotic solvents.

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Journal of the American Chemical Society vol. 108, no. 17, 20 August 1986, USA pages 5282 - 5287; S. Marburg et al.: "Bimolecular Chemistry of Macromolecules:Synthesis of Bacterial Polysaccharide Conjugates with Neisseria meningitidis Membrane Protein"

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Description

It is often advantageous to solubilize polysaccharides in aprotic solvents to carry out reactions that would not be possible in protic media. Such reactions include those which activate polysaccharides using a reagent that is sensitive to water.

Marburg et al., U.S. Patent No. 4,695,624, describes the functionalization of polysaccharides for conjugation to proteins in order to prepare polysaccharide-protein conjugates, which are useful as vaccines. The polysaccharides must be covalently-modified prior to conjugation by first solubilizing them in aprotic (non-hydroxylic) solvents so that nucleophilic hydroxyl groups of the polysaccharides can react with electrophilic reagents.

Egan, et al. J. Amer. Chem. Soc., 1986, 108, 5282-5287 describes the adipic acid dihydrazide (AAD) functionalization of bacterial polysaccharide termini with a carbodiimide. A similar reaction with AAD-functionalized proteins would provide polysaccharide-protein conjugates, which also may be useful as vaccines. In order to avoid competing cyclophosphate formation from the O-phosphoryl isourea intermediate, the reaction must be carried out in aprotic media. AAD is a more reactive nucleophile in aprotic solvents than in water, and can effectively compete with intramolecular cyclization.

Previously, the solubilization of polyanionic bacterial polysaccharides in aprotic solvents was achieved by replacement of the alkali metal or alkali-earth metal cations with large hydrophobic cations such as tri- or tetra-alkylammonium, 1-azabicyclo-[2.2.2]-octane, and 1,8-diazabicyclo[5.4.0]undec-7-ene. Solubilization was preferably accomplished by passing the polysaccharide through a strong acid cation exchange resin in the tetralkylammonium form.

Solubilizing polysaccharides according to the method described in Marburg et al., and Egan et al., i.e. by passing the polysaccharide through a strong acid cation exchange resin, requires extensive processing time, numerous operations and large quantities of raw material. In addition, yields suffer because of binding of the polysaccharides to the resin.

A purpose of the present invention is to provide an efficient process for solubilizing polysaccharides in aprotic solvents, using precipitation as the means for cation exchange rather than passage through a cation exchange resin. Precipitation increases yield, and reduces processing time, the number of operations and the amount of raw material required to obtain a given amount of solubilized polysaccharide.

The present invention is a simple, efficient process for solubilizing polyanionic bacterial polysaccharides such as polyribosylribitol phosphate (PRP) in aprotic solvents. Polysaccharides solubilized in aprotic solvents are particularly suited for conjugation to proteins.

The process takes advantage of the fact that polyanionic bacterial polysaccharides are often isolated as their calcium salts. The process renders bacterial polysaccharides soluble in aprotic solvents by exchanging the calcium counterion of a polysaccharide calcium salt for tetra-n-alkylammonium ion, preferably, tetra-(C_1-C_{12}) alkylammonium ion, more preferably tetra-n-butylammonium ion.

The exchange is accomplished by reaction of the polysaccharide with an acid, preferably oxalic, that forms an insoluble salt with calcium. The solution of polysaccharide is then titrated with tetra-n-alkylammonium hydroxide to give the tetra-n-alkylammonium polysaccharide salt. Alternatively, the acid and tetra-n-alkylammonium hydroxide are pre-mixed, and the tetra-n-alkylammonium polysaccharide salt is obtained directly. The precipitate of the insoluble calcium salt is removed by centrifugation or filtration. A solution of the tetra-n-alkylmmonium polysaccharide salt in an aprotic solvent is obtained by lyophilization to remove water and dissolution of the solid in the aprotic solvent, or, preferably, by displacement with the aprotic solvent by distillation.

In one embodiment of the invention, polysaccharide is solubilized in an aprotic solvent by adding an aqueous solution of a calcium salt of a polysaccharide to a suitable amount of aqueous oxalic acid, adjusting the pH of the resulting mixture to 7 with the addition of a tetra-n-alkylammonium hydroxide to replace the calcium counterion with tetra-n-alkylammonium ion and enhance solubility of the polysaccharide in aprotic solvents, removing the calcium oxalate, and replacing the water with the aprotic solvent.

In an alternative embodiment, polysaccharide is solubilized in an aprotic solvent by adding a suitable amount of an aqueous solution of tetra-n-alkylammonium salt, adjusted to a pH of 4 with an appropriate acid, to an aqueous mixture of a calcium salt of a polysaccharide, adjusting the pH of the resulting mixture to 7 with tetra-n-alkylammonium hydroxide to replace the calcium counterion with tetra-n-alkylammonium ion and enhance solubility of the polysaccharide in aprotic solvents, removing the precipitate, and replacing the water with the aprotic solvent.

In another alternative embodiment, polysaccharide is solubilized in an aprotic solvent by adding a suitable amount of an aqueous solution of tetra-n-alkylammonium salt, adjusted to a pH of 7 with an appropriate acid or salt, to an aqueous mixture of a calcium salt of a polysaccharide to replace the calcium counterion with tetra-n-alkylammonium ion and enhance solubility of the polysaccharide in aprotic solvent, removing the precipitate, and replacing the water with the aprotic solvent.

Polysaccharides that can be solubilized in aprotic solvents by the process of the invention may be any bacterial polysaccharides with acid groups. Teichoic acid-like polysaccharides and those containing neu-